

Root secretion stimulating ash growth in larch-ash mixed forest¹

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Abstract Allelopathic effect of larch (*Larix gmelini*) on the ash growth (*Fraxinus mandshurica*) was studied in artificial cultivation tests. The results revealed that the larch root secretion obviously stimulated the ash growth. In order to determine the main stimulation allelochemicals, the chemical composition was analyzed. By contrasting the contents of carbohydrate and aminoacid in root secretion of larch and ash, it was concluded that the carbohydrate and aminoacid were not important stimulation allelochemicals. The organic acid and other components in root secretion of larch and ash were analyzed by GC and GC-MS analysis. The sand culture tests were carried out with selected model compounds. The results showed that benzenecetic acid, benzenepropionic acid and phenolic acids in root secretion of larch were the main stimulation allelochemicals.

Key words: Larch, Ash, Allelopathy, Root secretion, Mixed forest

Introduction

Reported as early as 1920s, the growth of ash could be increased as ash was mixed with larch. However, the mechanism of allelopathic effect between larch and ash has not been clearly yet. The allelopathic effects in mixed forest were generally regarded as following four ways: volatile matters, root secretion, stem leaching, and litter decomposition matters (Zhai 1993). This paper presented the studies on the effect of root secretion, focused on those stimulation allelochemicals that can increase the growth of ash

Test methods

Sand culture test

The sand was sieved with 2 mm mesh screen and washed out the mud, soaked in 5% HCl solution about 1h. Then the sand was washed with water till it was neutral. The larch seedlings of 2-year-old and the ash seedlings of 1-year-old were used for experimental materials. The seedling roots were sterilized with NaClO solution before planting in the sand pots. Hoagland culture solution was used in sand

culture (Plant Physiology Society of Shanghai 1985).

Analysis of carbohydrate and aminoacid

The anthrone paper chromatography and the anthrone spectrophotometry were used to qualitative analysis and quantitative analysis of carbohydrate (Harborne 1984). The aminoacids were measured with aminoacid autoanalyser. The analyzed sample was the leachate of sand culture.

Analysis of organic acid and other compounds

After the roots of ash and larch rinsed and immersed in distilled water for 48h, the water extract containing the root secretion was obtained. The extract of root secretion was treated with vacuum condensation and vacuums freeze drying, for obtaining dried root secretion. After the dried root secretion with methanol, the methanol solution was treated with CH₂N₂ ether solution to carry out methylation. After adding water, the methylated solution was extracted three times with ether. After the combined ether extracts were then dried in water-bath, the remains were dissolved with CH₂Cl₂, and the methylated root secretions were analyzed by GC and GC-MS methods.

GC condition: GC-9A gas chromatograph, SE-52 capillary column, 50 m×0.25 mm, carrier gas N₂, injection temperature for 250°C, column temperature in 100-240°C, 8°C/min, FID.

GC-MS condition: Qmass-910 GC-MS analyzer, DB-5 capillary column, 30 m×0.25 mm, carrier gas with He, injection temperature of 250°C, column temperature of 100-240 °C, 8 °C per min, temperature of ion source in 200°C, EI, 70eV.

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Model compounds test

The selected model compounds were, respectively added to the Hoagland culture solution (25 mg/L), and used to do sand culture tests.

Results

Sand culture test

The effect of larch root secretion on the growth of ash is observed in sand culture test that ash and larch are mixed planted. The new branch growth of per ash seedling is shown in Table 1.

Table 1. The result of sand mixture culture of larch and ash

Culture condition	Test period /d	Net growth /mm ³	Increase rate (%)
Ash and larch in mixture	54	396	135.7
	93	442	53.5
Pure ash	54	168	
	93	288	

The growth of ash is improved clearly by mixture planting with larch. The improving effect is obviously exerted by the root secretion of larches.

Carbohydrate and amino acid

According to anthrone chromatography analysis, carbohydrate was in the leachates of both ash and larch sand culture tests. The carbohydrate content in the root secretion of larch is 8.5-9.0 mg/L, which is 10-11 mg/L lower than that of ash. Therefore, we conclude that carbohydrate is not the stimulation allelochemicals of improving the growth of ash.

Dissociative amino acid in the leachates from both ash and larch sand culture is measured with amino acid auto-analyser (Fig. 1-2). The results in Table 2 reveal that dissociative amino acids in the root secretion of ash are more abundant in either variety or quantity than that of larch. Therefore, dissociative aminoacids in the root secretion of larch are not the stimulation allelochemicals of improving the ash growth

Table 2. The content of amino acids in the leachate of sand culture (mg·L⁻¹)

Aminoacids	Larch	Ash
Aspartic acid	3	5
Glutamate	3	5
Serine	10	6
Glycocol	-	30
Hercynine	-	2
Arginine+threonine	-	48
Alanine+proline	1	4
Picramic acid	2	6
Valine	2	4
Methionine	1	4
Cystine	-	2
Isoleucine	2	4
Leucine	2	4
Phenylalanine	1	4
Lysine	1	2

Organic acid and other compounds

Fig.1 and Fig.2 are total ion current chromatogram of methylated derivative of ash and larch root secretion, respectively. The results of GC determination and GC-MS identification for these methylated derivatives are shown in Table 3 and Table 4.

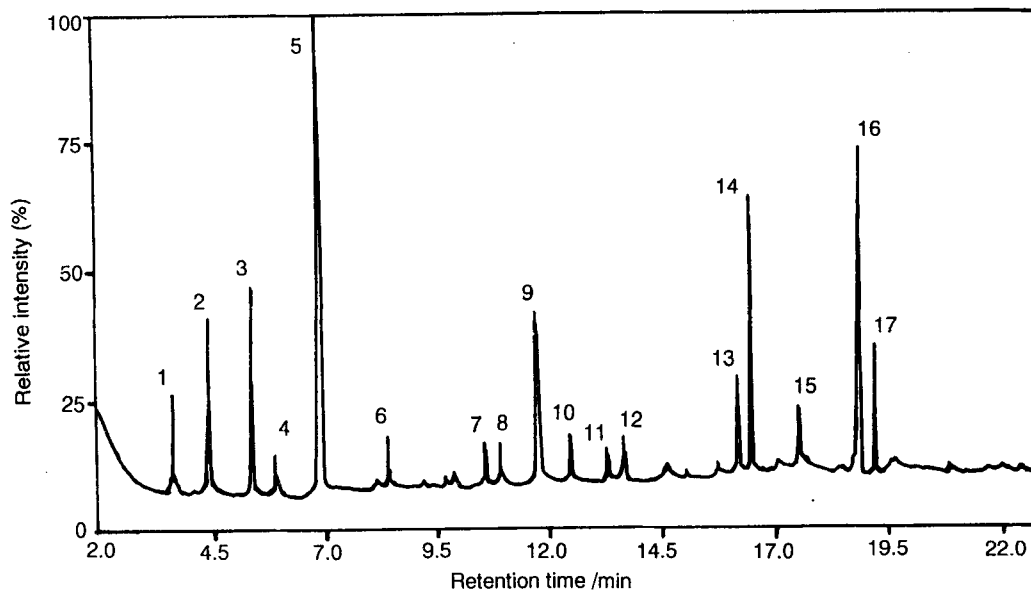


Fig. 1. Total ion current chromatogram of methylated derivative of larch root secretion

1. Dimethyl succinate; 2. Methyl benzoate; 3. Methyl benzenecacetate; 4. Methyl octanate; 5. Methyl benzenepropionate; 6. Methyl hydroxybenzenepropionate; 7. Methyl 4-methoxybenzenepropionate; 8. Dimethyl nonandiate; 9. Methyl 3,4-dimethoxybenzoate; 10. Dimethyl decanediante; 11. Methyl tetradecanate; 12. Metyl 3,4-dimethoxybenzenepropionate; 13. Methyl palmitoleate; 14. Methyl palmitate; 15. Methyl margarate; 16. Methyl oleate; 17. Methyl stearate.

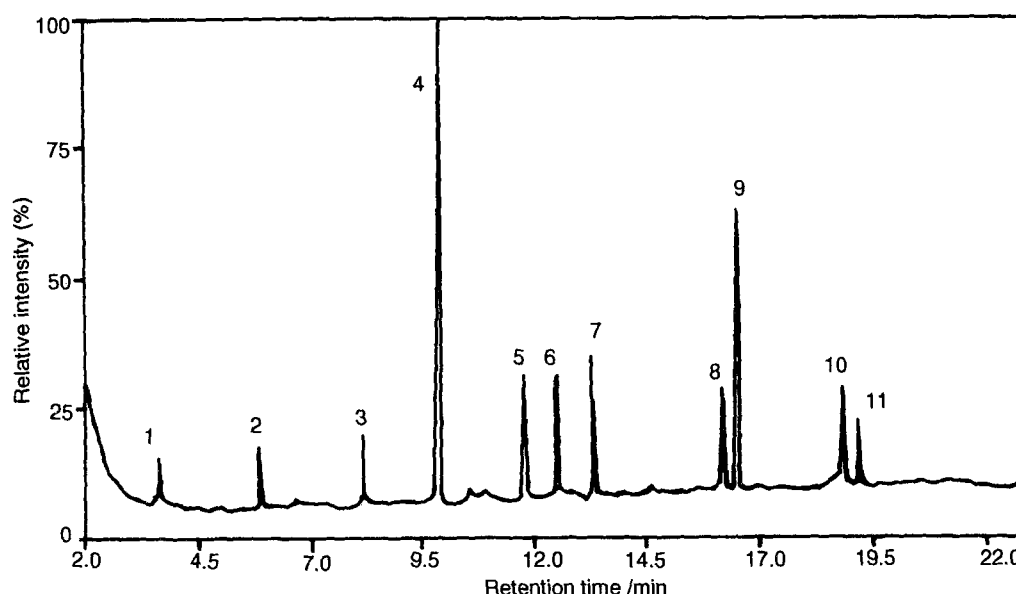


Fig. 2. Total ion current chromatogram of methylated derivative of ash root secretion

1. Dimethyl succinate; 2. 2,4-Dimethyl-2,4-heptadienol; 3. 4-Methoxybenzeneethanol; 4. 3,4-Dimethoxybenzeneethanol; 5. Methyl 3,4-dimethoxybenzoate; 6. Dimethyl decanediate; 7. Methyl 3,4-dimethoxybenzenepropionate; 8. Methyl palmitoleate; 9. Methyl palmitate; 10. Methyl oleate; 11. Methyl stearate.

According to the results shown in Table 3 and Table 4, the similarities and differences between ash and larch in the chemical composition of root secretion are summarized as follows:

(1) In the ash root secretion, there is a large amount of aromatic alcohols, which is not found in larch root secretion.

(2) The phenolic structure contains in root secretion

of ash and larch.

(3) The quantity of aromatic acids in the larch is higher than that of ash, and benzenecetic acids contained in the larch root secretion, which is unfound in the ash root secretion.

(4) The content of dinary acid in two kinds of root secretion is almost equal (Table 5).

Table 3. The chemical composition of methylated derivative of larch root secretion

No.	Compounds	Molecular formula	Molecular weight	Content (%)
1	Dimethyl succinate	$C_6H_{10}O_4$	146	3.74
2	Methyl benzoate	$C_8H_8O_2$	136	6.42
3	Methyl benzenecetate	$C_9H_{10}O_2$	150	8.21
4	Methyl octanate	$C_9H_{18}O_2$	158	3.16
5	Methyl benzenepropionate	$C_{10}H_{12}O_2$	164	21.05
6	Methyl α -hydroxybenzenepropionate	$C_{10}H_{12}O_3$	180	1.56
7	Methyl 4-methoxybenzenepropionate	$C_{11}H_{14}O_3$	194	1.84
8	Dimethyl nonandiate	$C_{11}H_{20}O_4$	216	1.26
9	Methyl 3,4-dimethoxybenzoate	$C_{10}H_{12}O_4$	196	6.26
10	Dimethyl decanediate	$C_{12}H_{22}O_4$	230	1.95
11	Methyl tetradecanate	$C_{15}H_{30}O_2$	242	2.00
12	Methyl 3,4-dimethoxybenzenepropionate	$C_{12}H_{16}O_4$	224	2.37
13	Methyl palmitoleate	$C_{17}H_{32}O_2$	268	3.53
14	Methyl palmitate	$C_{17}H_{34}O_2$	270	8.90
15	Methyl margarate	$C_{18}H_{36}O_2$	284	2.11
16	Methyl oleate	$C_{19}H_{36}O_2$	296	17.11
17	Methyl stearate	$C_{19}H_{38}O_2$	298	8.32

Table 4. The chemical composition of methylated derivative in ash root secretion

No.	Compounds	Molecular formula	Molecular weight	Content (%)
1	Dimethyl succinate	C ₈ H ₁₀ O ₄	146	0.68
2	2,4-Dimethyl-2,4-heptadienal	C ₉ H ₁₄ O	138	1.58
3	4-Methoxybenzeneethanol	C ₉ H ₁₂ O ₂	152	2.28
4	3,4-Dimethoxybenzeneethanol	C ₁₀ H ₁₄ O ₃	182	37.51
5	Methyl 3,4-dimethoxybenzoate	C ₁₀ H ₁₂ O ₄	196	6.45
6	Dimethyl decanediate	C ₁₂ H ₂₂ O ₄	230	7.47
7	Methyl 3,4-dimethoxybenzenepropionate	C ₁₂ H ₁₆ O ₄	224	9.71
8	Methyl palmitoleate	C ₁₇ H ₃₂ O ₂	268	6.61
9	Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	12.72
10	Methyl oleate	C ₁₉ H ₃₆ O ₂	296	7.87
11	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	6.91

Model compound test

According to the result of chemical analysis, model compounds are chosen, and sand culture tests are carried out. The structural types of all the compounds in the root secretion are almost contained in the model compounds.

Table 5. Result of model compound test

Model compound	Net growth of ash /cm ³	Increase rate (%)
Succinic acid	3.35	55.81
Catechol	1.54	-28.37
Benzoic acid	1.95	-9.30
Benzeneacetic acid	3.01	40.00
Benzenepropionic acid	2.44	13.49
Stearic acid	1.48	-31.16
p-Hydroxybenzoic acid	2.57	19.53
Phenethyl alcohol	2.23	3.72
Control	2.15	

Note: Net growth of ash means growing-yield of per tree

The results shown in Table 5 reveal that phenols and higher content of fatty acids restrain to the growth of ash. Aromatic acids of different structures have different allelopathic effect: benzoic acid has weak inhibitor action, and benzenepropionic acid in some

extent can improve the growth of ash, while benzeneacetic acid exerts strong stimulative effect. It is interesting that phenolic acids show stimulative effect, although phenols and benzoic acid are inhibitor allelochemicals. Aromatic alcohols have no obvious effect upon the growth of ash.

The real action of water-soluble aliphatic dicarboxylic acid on the growth of ash is not obvious, although its contents are lower and these dicarboxylic acids have strong stimulative allelopathy.

According to the results in Table 4 and Table 5, benzeneacetic acid, benzenepropionic acid and phenolic acids are the main stimulation allelochemicals.

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